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LETTER TO THE EDITOR

Transfection of Restrictionless *Escherichia coli* by Bacteriophage T7 DNA: Effect of *In vitro* Erosion of DNA by λ Exonuclease

T7 bacteriophage infects with equal efficiency restriction-proficient *Escherichia coli* K12 cells and the restriction-deficient mutants. To the contrary, the purified phage DNA transfects wild-type cells at a very low efficiency (10^{-9} plaques/genome equivalent). Mutations in the recB recC (exonuclease V) and sbcB (exonuclease I) loci increase the transfecting efficiency tenfold. A 1000-fold increase is obtained with cells deficient in restriction. No further increase is observed in hosts carrying both sets of mutations. The transfecting activity of the DNA on restriction-deficient hosts increases another 20-fold (up to 4×10^{-5} plaques/genome equivalent) by complete erosion of the redundant regions of DNA with λ exonuclease, both in *rec*$^+$ and *rec*B *rec*C *sbc*B genotypes. Circles and linear oligomers arising from the annealing of eroded DNA show the same transfecting activity as the unannealed monomers. The terminal redundancy of the genome, as measured by the onset of annealability of eroded molecules, was found to comprise 50 to 100 base-pairs.

The T7–*Escherichia coli* transfection system is experimentally attractive in the light of the well-defined genetic program carried by the T7 genome (Studier, 1972). Benzinger *et al.* (1975) have reported the transfection of *E. coli* spheroplasts with an efficiency of $2{\cdot}5 \times 10^{-7}$ pge† for native T7 DNA and of $1{\cdot}1 \times 10^{-6}$ for denatured DNA. We have been able to transfect *E. coli* cells rendered competent by $CaCl_2$ treatment (Mandel & Higa, 1970). Our results are summarized in Table 1.

T7 phage can efficiently infect *E. coli* K12 and B strains, regardless of its previous host (a result in agreement with published data, Eskridge *et al.*, 1967). Its DNA, to the contrary, can transfect K12 hosts with wild-type restriction and recombination characteristics only very inefficiently (10^{-9} pge). Strains with inactive recBC nuclease can be transfected 10 times better, while the strains lacking restriction nuclease are 1000 times more transfectable. Concurrent loss of both restriction and the recBC enzyme does not bring forward a further improvement in transfectability over the level obtained with restrictionless hosts.

An explanation for these results can be suggested on the basis of the recent findings of the effect of T7 infection on the restriction and the recBC enzymes of the host: the DNA injected from the T7 particles bypasses the host restriction–modification system by either (a) the simultaneous injection of an interfering protein or (b) its prompt intracellular synthesis, before the phage DNA could be degraded (Studier, 1975). Similarly, it has been shown that upon infection with T7 phage the host recBC gene product, exonuclease V, is also being inactivated (Wackernagel & Hermanns, 1974; Sakaki, 1974). Cell defenses having thus been overcome, the infecting DNA can pursue its density of producing new phage particles. In contrast, naked DNA, sensitive to the *E. coli* restriction system (Eskin *et al.*, 1973) is not able to cope with it during transfection, perhaps due to the absence of inhibitor (carried in the intact particles)

† Abbreviation used: pge, plaques per genome equivalent.

TABLE 1

Effect of recombination and restriction properties of the host on T7 DNA†

Strain	Relevant markers§	T7 (B) Infection (relative)	T7 (B) Transfection pge	T7 (K) Infection (relative)	T7 (K) Transfection pge	DNA/cell ratio‡
W3292 (B)[a]	—	<1·0>	N.D.	1·1	N.D.	
W5410 (C600)[b]	—	0·85	10^{-9}	1·0	10^{-9}	20–50
W3110 (K12)[c]	—	0·80	10^{-9}	<1·0>	N.D.	20–50
W5418 (K12)[d]	*rec*B	N.D.	10^{-8}	N.D.	N.D.	2–5
W5419 (K12)[d]	*rec*C	N.D.	10^{-8}	N.D.	10^{-8}	2–5
W5437 (K12)[d]	*rec*B *rec*C *sbc*B	0·50	10^{-8}	N.D.	10^{-8}	2–5
W5438 (C600)[b]	r^- m^-	0·85	10^{-6}	1·0	N.D.	0·01–0·4
W5446 (C600)[b]	r^- m^-	0·85	10^{-6}	N.D.	N.D.	0·01–0·4
W5445 (C600)[b]	r^- m^+	N.D.	10^{-6}	0·9	10^{-6}	0·01–0·4
W5449 (K12)[e]	r^- m^+ *rec*B *rec*C *sbc*B	N.D.	10^{-6}	0·9	10^{-6}	0·01–0·4

† DNA was extracted from the T7 phage grown on *E. coli* B (W3292) or on K12 host (W5437). Transfection was performed as described in the preceding paper. Results shown represent average of several experiments, variation in competence being corrected for by using λ DNA (which gave about 10^{-4} pge on all of the strains shown) as a standard transfecting agent.

‡ Linear response range, except for W5410 and W3110 where too few plaques were obtained to establish the correlation.

§ Additional markers for the strains shown here are the following:

[a] *mal.*

[b] F^- *thr*$^-$ *leu*$^-$ *thi*$^-$ *sup*E44 *lac*$^-$ *ton*A21.

[c] $F^-\lambda^s$.

[d] $F^-\lambda^s$ *thr*$^-$ *leu*$^-$ *arg*$^-$ *his*$^-$ *pro*$^-$ *thi*$^-$ *ara*$^-$ *lac*$^-$ *gal*$^-$ *mtl*$^-$ *xyl*$^-$ *str*^{R} *tsx*.

[e] As [d] above, but *thr*$^+$ *tryp*$^-$.

N.D., not determined.

or the failure of proper compartmentalization and/or timing of expression of its gene, consequent to the peculiar mode of entry. In addition to being attacked by the host restriction nuclease, the transfecting DNA is susceptible to the action of the recBC nuclease; this enzyme could act on the fragments resulting from the restriction of the phage DNA possibly because of the rapid inactivation of the T7 gene coding for the inhibitor of the recBC nuclease by the restriction endonuclease. Thus the strains W5418 and W5419, lacking a functional exonuclease V, can be transfected with a tenfold higher efficiency than the *rec*$^+$ W5410 and W3110. It is possible that this slightly increased transfecting activity is due to multiplicity reactivation of the restriction-produced DNA segments. The DNA : cell ratio in the experiments discussed here was 2:5. Lack of exonuclease I in addition to recBC enzyme has no effect on transfectivity (W5437). If the restriction nuclease has been inactivated by a mutation, transfecting DNA remains uncleaved, and can be expressed as well as protected from the degradation by the recBC enzyme (see strains W5438, W5446 and W5445). Consequently, the concomitant lack of both the restriction and the recBC nuclease activity should not and does not bring forth a further increase in the transfectability over cells lacking only the restriction nuclease (compare W5449 to the 3 above strains).

The stoichiometry of transfection of a $CaCl_2$-treated restrictionless *E. coli* strain with the T7 DNA is shown in Figure 1. The number of plaques is linearly proportional to the amount of DNA in the range of 0·5 to 20 ng per plate. The efficiency of transfection is close to 2×10^{-6} pge, a value about 10 times higher than the one obtained with spheroplasts and native T7 DNA (Benzinger *et al.*, 1975), but still about two orders of magnitude lower than that displayed on the same host by λ DNA. One of the structural differences, which we thought might account for unequal biological activity of T7 and λ DNA, is the presence of the sticky ends on the latter; if these are removed *via* repair with a DNA polymerase, the transfectivity of the λ DNA is lost (Strack & Kaiser, 1965). As the annealable ends can be created by an appropriate exonucleolytic treatment of T7 DNA (which is endowed with redundant duplex

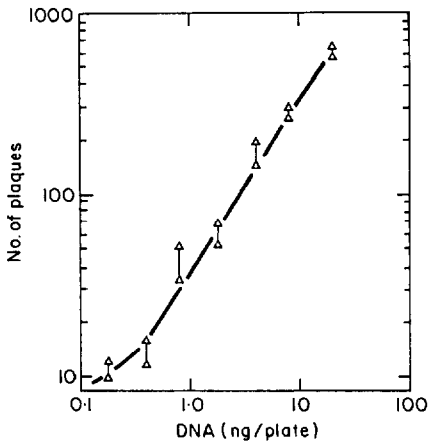

FIG. 1. Stoichiometry of transfection of $CaCl_2$-treated restrictionless *E. coli* cells with T7 DNA. T7 DNA was extracted from phage particles grown on *E. coli* B (W3292) and purified by differential centrifugation and repeated banding in CsCl density gradients. Restrictionless *E. coli* K12 (W5438) cells were brought to competence and transfected as described in the accompanying paper (Sgaramella *et al.*, 1976). 10^9 cells were spread on each plate.

terminal regions, Ritchie *et al.*, 1967), we have decided to study the effects of λ-exonuclease erosion† on the biological activity of T7 DNA. Such a treatment has been shown to be effective in increasing the transfecting activity of the P22 DNA which also has redundant ends (Sgaramella *et al.*, 1976).

The removal of nucleotides from T7 DNA by saturating amounts of λ exonuclease shows no lag and proceeds linearly with time at a rate close to 80 nucleotides per strand per hour at optimal pH and 0°C (Fig. 2(a)); at pH 7 and 20°C this rate is

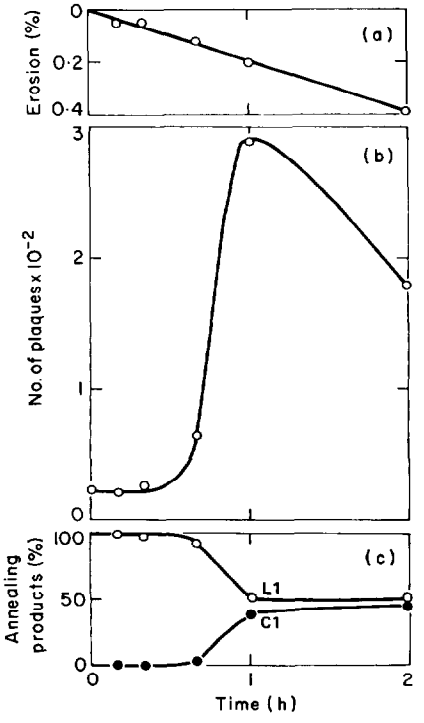

FIG. 2. Effect of λ exonuclease erosion on the transfecting activity and annealing properties of T7 DNA. (a) A 100-μl reaction mixture containing 60 mM-K-glycinate (pH 9·4), 4 mM-$MgCl_2$, 3 μg T7 DNA and 1·3 μg λ exonuclease was incubated at 0°C. 20-μl samples were withdrawn at the indicated times; 10 μl were used to determine the extent of erosion (Sgaramella *et al.*, 1976). The other 10 μl were diluted in SSC (SSC is 0·15 M-NaCl, 0·015 M-sodium citrate, pH 7·0) and used in part for transfection at a DNA concentration of 1 ng/plate (b). The rest, after further dilution to 1 μg/ml in 2 × SSC, was annealed at 65°C for 30 min and submitted to electron microscopy analysis (c).

about 90 nucleotides per strand per minute (Sgaramella *et al.*, 1976). The effect of erosion on the transfecting activity of T7 DNA in a r^- rec^+ genotype of *E. coli* is shown in Figure 2(b). With the removal of 0·1 to 0·2% of nucleotides, the transfectivity abruptly rises about 20-fold, reaching an efficiency of about 4×10^{-5} pge, a value close to that displayed by λ DNA (10^{-4} pge). An essentially identical activation was seen in a strain of the r^- *recB recC sbcB* genotype. Increase in transfectivity is paralleled by the ability of eroded molecules to form hydrogen-bonded circles

† See accompanying paper (Sgaramella *et al.*, 1976) for explanation of the term, erosion.

(Fig. 2(c)). This points to the dependence of augmentation of biological activity on the annealability of the T7 DNA molecules, a result expected in view of the known behavior of λ and P22 DNA (Strack & Kaiser, 1965; Sgaramella *et al.*, 1976).

Spurred by the concurrence of the increase of biological activity and the annealability of T7 DNA molecules (Fig. 2), we have investigated whether the different molecular species, resulting from annealing λ exonuclease-eroded T7 DNA, contribute differentially to the increase of transfectivity. Two approaches were followed.

(1) T7 DNA was eroded to 0·3% (the resulting increase in transfecting activity being about 15-fold) and annealed at high (25 μg/ml) and low (2 μg/ml) concentration, in order to favor intermolecular oligomerization or intramolecular circularization, respectively (Davidson & Szybalski, 1971). Electron microscopy (Sgaramella *et al.*, 1976) of the annealed mixtures revealed that, as expected, in the former case the majority of the molecules had undergone linear oligomerization, while in the latter the monomeric circles were preponderant (Table 2). The transfecting activities of the two annealing mixtures were found to be essentially the same, a 15% difference being within the experimental error.

TABLE 2

Transfecting activity and molecular species of eroded annealed T7 DNA†

DNA annealing concentration (μg/ml)	Linear monomers (%)	Circular monomers (%)	Linear oligomers (%)	Other‡ (%)	Transfectivity§ (pge)
2	30	60	5	5	3×10^{-5}
25	35	10	50	5	$3{\cdot}5 \times 10^{-5}$

† Erosion to 0·3% and annealing were done as described in the legend to Fig. 2.

‡ Circular dimers, "theta" and "sigma" shaped molecules.

§ Transfection was performed at the concentration of annealed DNA of 1 ng per plate as described in the preceding paper (Sgaramella *et al.*, 1976).

(2) DNA was eroded to 1·2% (with an enhancement of transfectivity close to tenfold) and annealed at a concentration of 10 μg/ml. The separation of annealing products was achieved by neutral sucrose sedimentation (Sgaramella *et al.*, 1976). Fractions traveling with increasing velocity were pooled and analyzed (a) by electron microscopy, (b) for their transfecting activity. Table 3 summarizes the result of this analysis: it is apparent that the transfecting activities displayed by the different fractions ranged in a narrow interval although their relative content in monomeric circles, oligomeric linears and circles is rather different.

Activation of P22 DNA upon erosion is correlated with the ability of eroded molecules to form hydrogen-bonded circles which escape degradation by the recBC nuclease (Sgaramella *et al.*, 1976). The same explanation does not hold true for the T7 DNA: although, as is the case for P22 DNA, the activation is brought forward concurrently with the ability of molecules to anneal, no specific structure is endowed with a particularly high transfecting activity. Furthermore, the transfectivity of T7 DNA is unaffected by the *rec* genotype of the host.

The activation of T7 DNA upon erosion might be due to various reasons; an attractive explanation is that the eroded molecules can undergo, *via* their exposed complementary single-stranded ends, a rapid intracellular reassembling into structures

TABLE 3

Transfecting activity of annealed T7 DNA species fractionated by sedimentation†

Relative sedimentation velocity	Frequency				Transfecting efficiency (pge $\times 10^5$)	Relative transfectivity
	Linear monomers (%)	Circular monomers (%)	Linear oligomers (%)	Circular oligomers (%)		
<1>	93	1	6	0	2·1	<1>
1·1	79	21	0	0	1·8	0·86
1·3	34	60	6	0	1·7	0·81
1·4	32	4	65	0	1·7	0·81
1·6	47	0	24	30	1·5	0·71

† DNA was eroded and annealed as described in the legend to Fig. 2. Separation was obtained by sedimenting the sample through a 5% to 20% sucrose gradient in a SW 50.1 rotor at 49,000 revs/min for 2 h. Different fractions were pooled, dialyzed against 10 mM-Tris·HCl (pH 7·6) and analyzed for transfectivity or by electron microscopy. Close to 100 molecules were scored from each pool for the electron microscopy analysis.

intermediary in the life cycle of the T7 phage (Schlegel & Thomas, 1972; Wolfson *et al.*, 1972). The absence of the effect of the recBC nuclease on the activation of the DNA is not surprising as the T7 genome (unlike P22) is able to inactivate the enzyme (Wackernagel & Hermanns, 1974; Sakaki, 1974).

A side issue, interesting to note here, is that the data presented in Figure 2(c) indicate that the redundant regions of T7 DNA comprise 35 to 70 base-pairs, judged by the ability of T7 DNA eroded to 0·1 to 0·2% to form hydrogen-bonded circles (the T7 DNA molecule is 35,000 base-pairs long, Studier, 1972). Taking into account that annealing is not more extensive than about 70% (Table 2), at most 30% of molecules might have escaped erosion; the repetitive regions could thus be at most 50 to 100 base-pairs long. This value is substantially lower than the 250 base-pairs measured previously (possibly on a different strain of T7) by a different method (Ritchie *et al.*, 1967) and fits better the mode of replication of T7 DNA proposed by Watson (1972), being closer to the length of the RNA initiators of DNA replication (Hirose *et al.*, 1973).

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